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SHORT COMMUNICATION

EFFECTS OF α_2-ADRENOCEPTOR ANTAGONISTS AND IMIDAZOLINE$_2$-RECEPTOR LIGANDS ON NEURONAL DAMAGE IN GLOBAL ISCHAEMIA IN THE RAT

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SUMMARY

1. In the present study the neuroprotective effects of 3 mg/kg idazoxan, an α_2-adrenoceptor antagonist and imidazoline$_2$-receptor (I_2-receptor) ligand, 3 mg/kg methoxyidazoxan, a specific α_2-adrenoceptor antagonist, and 0.6 and 3 mg/kg BU224, a selective I_2-receptor ligand, were evaluated following 10 min of global ischaemia in rats.

2. Neuronal cell counts in the CA1 region of the hippocampus 8 days postischaemia indicated 46–96% cell loss compared with control ($P<0.001$) and a 320% increase in [^{3}H]-PK11195 binding ($P<0.001$) used as a marker of gliosis. No significant neuroprotective effect could be detected on these markers of neuronal damage in the active treatment groups. In a subset of idazoxan-treated rats, neuronal loss and gliosis was minimal.

3. Mean body temperature over 3 h postischaemia was lower in idazoxan-treated rats than in the other treatment groups ($P<0.001$) and there was a correlation between mean body temperature and cell counts ($P<0.01$) and mean body temperature and gliosis in this group ($P = 0.057$).

4. These results indicate that at the doses used neither BU224 nor methoxyidazoxan are neuroprotective in this ischaemia model and they raise the possibility that any neuroprotective effect of idazoxan may be related to hypothermic effects of the drug.

Key words: BU224, global ischaemia, idazoxan, methoxyidazoxan, neuroprotection.

INTRODUCTION

The α_2-adrenoceptor antagonist idazoxan, which also has activity at the I_1- and I_2-subtypes of the imidazoline receptor (I-receptor),[1] has been shown to reduce the extent of neuronal cell loss in the CA1 region of the hippocampus following global ischaemia.[2,3] This effect was attributed to the α_2-adrenoceptor antagonist properties of the drug; however, it was subsequently shown that both idazoxan and the α-adrenoceptor agonist rilmenidine (which also has activity at the I-receptor) reduced the extent of focal ischaemic infarction, whereas a selective α_2-adrenoceptor antagonist was without effect.[4] It was concluded that an interaction with I-receptors may mediate the neuroprotective effects of idazoxan and rilmenidine.

Recently, a new imidazoline drug, BU224, has been developed that has nanomolar affinity for I_2-receptors and an I_2/α_2 affinity ratio of >3000;[1] however, the neuroprotective properties of this compound have not been tested. In the present study, the effects of idazoxan, BU224 and the selective α_2-adrenoceptor antagonist methoxyidazoxan have been investigated in a model of transient global ischaemia in rats.

METHODS

Transient forebrain ischaemia was induced by the four-vessel occlusion technique.[5] Briefly, male hooded Wistar rats (250–300 g) were anaesthetized (methohexitone 32 mg/mL, amylobarbitone 60 mg/mL: 1 mL/kg, i.p.), the vertebral arteries were cauterized and clasps were placed around the carotid arteries. The external jugular vein was catheterized and the catheter was externalized at the back of the neck. On the following day, forebrain ischaemia was induced in conscious animals by occluding the carotid arteries for 10 min. Rats that did not lose their righting reflex (indicating that the vertebral arteries had not been closed adequately) had the carotid occlusion reversed after 1 min and were included as sham controls. After ischaemia, groups ($n = 7$–9) received either normal saline, idazoxan 3×1 mg/kg, methoxyidazoxan 3×1 mg/kg, BU224 3×1 mg/kg or BU224 3×0.2 mg/kg. Controls (<1 min ischaemia) received normal saline. Idazoxan (total dose 3 mg/kg) has previously been shown to reduce brain damage in focal and global ischaemia.[2–4] Methoxyidazoxan is 1.5–3-times more potent than idazoxan *in vitro*,[6,7] antagonizes central α_2-adrenoceptor-mediated effects on the cardiovascular system at doses of 0.5 mg/kg[8] but appears to be without effects at I-receptors at doses up to 10 mg/kg.[9,10] BU224 (10 mg/kg) produces effects similar to methoxyidazoxan on early response gene activity in rat brain, whereas 1 mg/kg does not[11] (A Gundlach, pers. comm., 1996). As BU224 has an I_2/α_2 affinity ratio of >3000,[1] the doses used in the present study should be active at the I_2-receptor site without activating α_2-adrenoceptors. Treatment was administered intravenously as three divided doses, immediately on reperfusion and then 1 and 2 h later. Body temperature was monitored for 180 min postischaemia using a rectal probe and was maintained at approximately 37.5°C using a heat lamp and mat.

Eight days following ischaemia, at a time when the delayed neuronal death following global ischaemia is maximal,[2,3,12] animals were anaesthetized and the brains were removed, frozen rapidly in liquid nitrogen and stored at −70°C. Coronal sections, 14 μm, were collected at bregma −3.30 mm[13] and thawed onto poly L-lysine-coated slides.

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Triplicate sections were stained with cresyl violet for Nissl substance and viable neuronal cells along a 1 mm length of the CA1 region of the hippocampus were counted. Adjacent triplicate sections were processed for quantitation of specific [^{3}H]-PK11195 binding.[14] [^{3}H]-PK11195 binds to the glial mitochondrial benzodiazepine binding site and is a particularly sensitive marker for ischaemic neuronal injury.[14]

[^{3}H]-PK11195 binding, the number of viable cells and mean body temperature over 3 h postischaemia were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's test comparing each treatment with sham.[15]

RESULTS

Histological evaluation of the CA1 region indicated a significant difference in the number of viable neuronal cells between

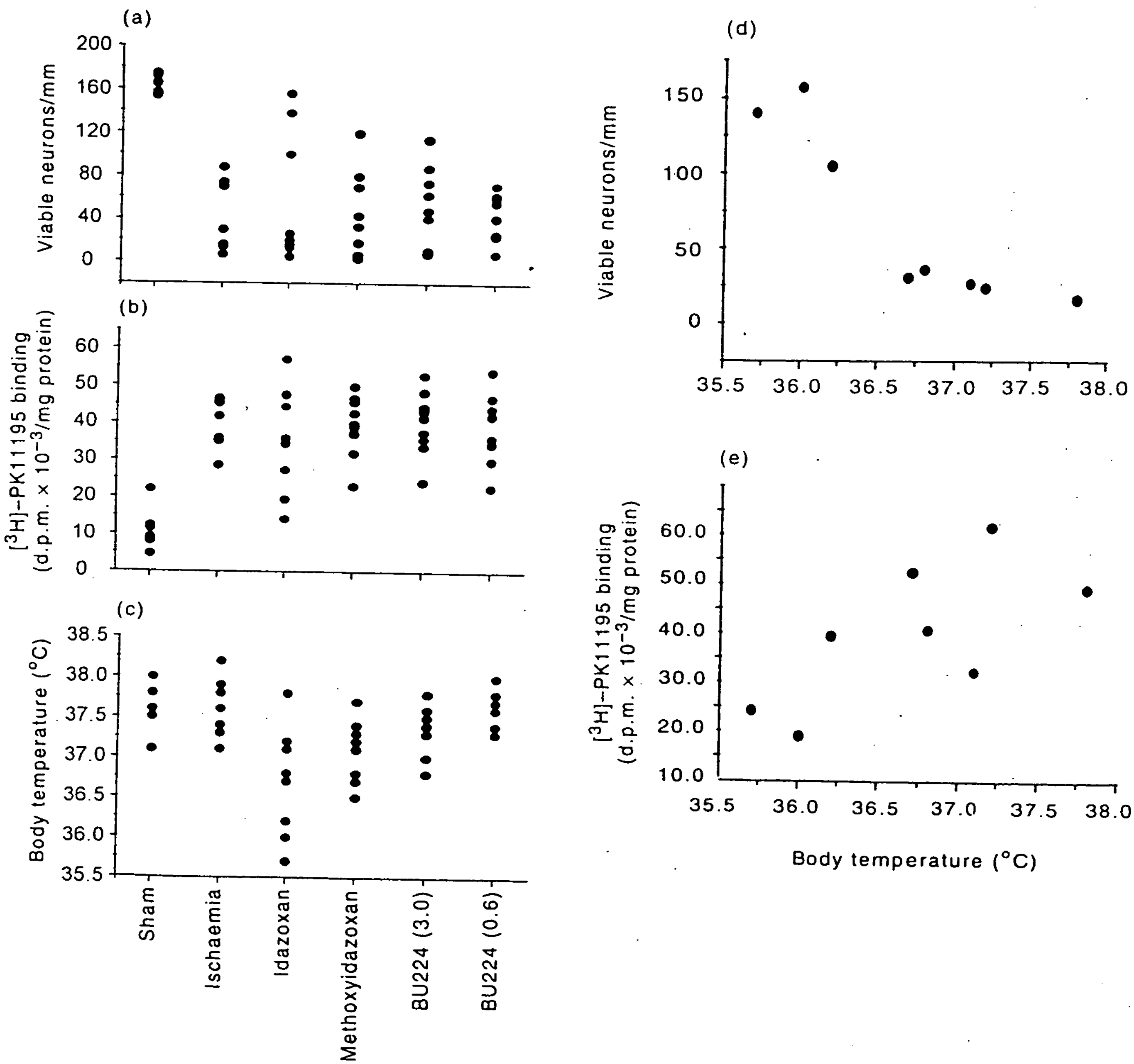

Fig. 1. (a–c) Effect of postischaemic administration of idazoxan (3.0 mg/kg), methoxyidazoxan (3.0 mg/kg) and BU224 (3.0 and 0.6 mg/kg) on neuronal cell loss (the number of viable neurons/mm CA1 pyramidal cell region) and (b) gliosis ([^{3}H]-PK11195 binding) occurring in the CA1 region of the hippocampus following 10 min of global forebrain ischaemia. (c) The effects of treatment on mean body temperature recorded over 3 h postischaemia are also shown. Individual values for each rat are shown ($n = 7$–9). BU224 (3.0), 3.0 mg/kg BU224; BU224 (0.6), 0.6 mg/kg BU224. (d,e) The correlation between mean body temperature and (d) neuronal cell loss ($r = -0.899$; $P<0.01$) and (e) [^{3}H]-PK11195 binding ($r = 0.694$; $P = 0.057$) in idazoxan-treated rats is shown.

groups (Fig. 1a; ANOVA, $F_{5,49}$; $P<0.001$). There was a 46–96% cell loss in the CA1 after 10 min ischaemia compared with sham control (Dunnett's test; $P<0.001$); however, no significant neuroprotective effect could be detected in the active treatment groups compared with ischaemic controls (Dunnett's test; $P>0.05$). The degree of gliosis in the CA1 region of the hippocampus was also group-dependent (Fig. 1b; ANOVA, $F_{5,49}$; $P<0.001$) with greater [^{3}H]-PK11195 binding in ischaemic controls compared with sham controls (Dunnett's test; $P<0.001$); however, there was no difference between the active treatment groups and the ischaemic controls (Dunnett's test; $P>0.05$). It was noted that in a subset of the idazoxan-treated rats, neuronal cell loss and [^{3}H]-PK11195 binding increases were minimal (Fig. 1a,b).

Body temperature was approximately 1°C lower immediately following ischaemia in both control and treatment groups (data not shown). In ischaemic controls and in methoxy-idazoxan- and BU224-treated rats, body temperature quickly returned to sham control levels and there was no overall treatment difference over the 3 h postischaemia between these groups (Fig. 1c; ANOVA, $F_{5,49}$; $P<0.001$; Dunnett's test $P>0.05$). In idazoxan-treated rats, however, body temperature remained depressed and, over the 3 h postischaemia, was significantly below that of ischaemic controls (Dunnett's test $P<0.001$). Further analysis showed there was a correlation between mean body temperature and cell counts and between mean body temperature and [^{3}H]-PK11195 binding in idazoxan-treated rats (Fig. 1d,e), but not in BU224-treated groups (temperature *vs* cell counts, BU224 (3.0 mg/kg), r = −0.30, P = 0.43; BU224 (0.6 mg/kg), r = −0.45, P = 0.23; temperature *vs* [^{3}H]-PK11195, BU224 (3.0 mg/kg), r = 0.25, P = 0.52; BU224 (0.6 mg/kg), r = 0.11, P = 0.78). In the methoxyidazoxan-treated group, there was no correlation between mean body temperature and cell counts (r = 0.11, P = 0.78), but mean body temperature was correlated with [^{3}H]-PK11195 binding (r = 0.72, P = 0.03).

DISCUSSION

In the present study the selective α_2-adrenoceptor antagonist methoxyidazoxan had no neuroprotective effect in the four-vessel occlusion model of forebrain ischaemia. This result supports the findings of Maiese *et al.*,[4] who demonstrated that a selective α_2-adrenoceptor antagonist had no effect in a focal ischaemia model, and argues against involvement of the noradrenergic system in modulating ischaemic neuronal damage through this receptor system. The selective I_2-receptor ligand BU224 also had no neuroprotective effect in the present study. The results do not support a role for the I_2-receptor in modulating ischaemic damage; however, future studies with agents known to be agonists or antagonists at the I_2-receptor site will be required to answer this question.

The neuroprotective effect of idazoxan in the present study was equivocal. Based on a group comparison, idazoxan treatment had no significant effect on ischaemic damage in contrast to reports that similar doses of idazoxan protected against hippocampal cell loss after global ischaemia[2,3] and reduced infarction areas after focal ischaemia.[4] In the present study, however, the greater spread in neuronal damage and gliosis accompanying ischaemia in the idazoxan-treated group was correlated with postischaemic hypothermia. Hypothermia is well established as having a neuroprotective effect in cerebral ischaemia[16] and even mild temperature drops (2–3°C) cause significant neuroprotection.[17] It is noteworthy that in idazoxan-treated animals in an earlier study[2] there was also a variability in the neuroprotective effect of the drug from <5 to >80% and body temperature was only monitored for 15 min post-ischaemia. At ambient temperature, with body temperature not regulated, idazoxan administration (3×1 mg/kg, i.v.) reduced average body temperature to 34.8±0.2°C compared with 37.1±0.1°C in control rats (EL Conway, unpubl. obs., 1996). It is possible that the variability in the neuroprotective effect of idazoxan may be related, in part, to this previously unreported hypothermic action. This action could be mediated through 5-HT_{1A} receptors as idazoxan has recently been reported to have agonist activity at 5-HT_{1A} receptors *in vitro* with an α_2/5-HT_{1A} affinity ratio of approximately 40.[18] 5-Hydroxytryptamine$_{1A}$ receptor agonists are potent hypothermic agents.[19] It is not known whether methoxyidazoxan or BU224 have activity at 5-HT_{1A} receptors.

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